NEUROSCIENCE

A long wait to refuel

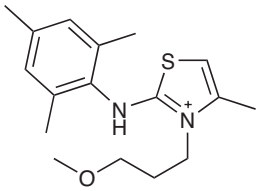
Neuron **76**, 511–517 (2012)

Neurotransmitters such as glutamate are released at synapses by exocytosis, followed by endocytosis to retrieve the vesicles and their refilling with neurotransmitter for recycling and continued synaptic transmission. The vesicle refilling speed has been predicted to be on the order of milliseconds. However, when monitored in isolated or reconstructed vesicles, the speed of glutamate uptake is upwards of 10 min. To look at this more closely in a physiological context, Hori and Takahashi monitored vesicle refilling kinetics at living synapses, where intravesicular glutamate could be washed out of nerve terminals, and refilling was monitored by introducing caged glutamate and measuring the recovery of excitatory postsynaptic currents. From these experiments, the authors concluded that uncaged glutamate was taken up via H^+-ATPase and the glutamate transporter VGLUT, with a maximal refilling time constant of 15.1 sec—much faster than in isolated or reconstructed vesicles and consistent with the idea that most vesicles are fully filled during recycling. As the refilling rates measured here do not support fast reuse of recycling vesicles consistent with a kiss-and-run (subsecond) mode of endocytosis, the results suggest that this fast endocytosis may contribute to balancing vesicular and terminal membranes rather than to synaptic transmission. *MB*

SIGNALING

Selecting the second messenger

Proc. Natl. Acad. Sci. USA **109**, 18613–18618 (2012)

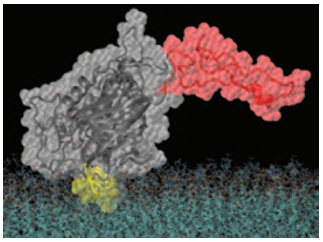


Exchange protein directly activated by cAMP (EPAC), a guanine nucleotide exchange factor (GEF), is one of the targets directly downstream of the second messenger cAMP. Distinguishing signals mediated by EPAC from another target protein kinase A (PKA) is challenging. Tsalkova *et al.* now report a small-molecule inhibitor of EPAC2. The authors performed a targeted high-throughput screen, looking for compounds that inhibit EPAC GEF activity. Two (ESI-05 and ESI-07) of the seven hit compounds were selective for EPAC2 over EPAC1 *in vitro* and in cells, showing dose-dependent activity with apparent IC_{50} values in the low micromolar range. Neither compound inhibited PKA activity *in vitro* or in cells. To determine the molecular basis for the compounds' selectivity for EPAC2, the authors performed deuterium exchange MS, which allowed them to map regions of lower solvent exposure in response to ESI-07. These data in conjunction with published crystal structure data allowed them to propose a model where ESI-07 binds at the interface of the two cAMP-binding domains, locking EPAC2 in an autoinhibited conformation. This mechanism would not be effective for EPAC1 because it has only one cAMP-binding domain. Although the mechanistic details remain to be confirmed, ESI-05 and ESI-07 are the first chemical probes shown to be selective for EPAC2. *AD*

MEMBRANES

PTEN heeds the charge

J. Struct. Biol. **180**, 394–408 (2012)



MATHIAS LÖSCHE

PTEN is a lipid phosphatase that hydrolyzes PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2, and as such, it controls many cellular signaling events, including proliferation and apoptosis. PTEN localization to the inner leaflet of the plasma membrane is regulated by its own phosphorylation and through interaction with its substrate and anionic lipids such as phosphatidylserine. To examine the changes in PTEN conformation with binding to the membrane, Shenoy *et al.* performed neutron reflectometry experiments on full-length PTEN adsorbed to planar model membranes. Molecular dynamics simulations were used to corroborate the neutron reflectometry results and to refine the experimentally determined structure. On the bilayer, the structure of the PTEN core is flattened against the membrane surface as compared to the published crystal structure of a truncated PTEN and the PTEN solution structure. The data also suggest that PS localizes PTEN via the CBR3 motif of its C2 domain at the membrane surface while subsequent interaction with the PtdIns lipid locks it into place. Moreover, the study reveals that the disordered C-terminal tail is repelled from the bilayer interface by electrostatic interactions. In contrast, the tail wraps around the C2 domain in solution. This high-resolution look at the changes in PTEN structure and membrane properties suggest how zipper-forming salt bridges between the C terminus and the C2 domain regulate PTEN activity by controlling its access to the membrane. *MB*

METABOLISM

A model merger

PLoS Comput. Biol. **8**, e1002750 (2012)

Metabolism is often considered only in terms of intracellular biochemical networks or across a complex organism, but a full understanding of human metabolism requires integration of these extremes. Toward this end, Krauss *et al.* explore how organismally focused, physiologically based pharmacokinetic (PBPK) models and genome-scale metabolic network models at the cellular scale could best be merged. Considering that PBPK models are often parsed into vascular, interstitial and intracellular spaces, the authors identified the intracellular space as a natural point of contact between the two models. The authors defined two ways in which a compound could affect the models: In indirect coupling, drugs or other inhibitors act as regulatory elements, influencing cellular metabolism without changing the availability of the compound in the PBPK model. In direct coupling, molecules from the PBPK model serve as substrates in the metabolic network model, causing changes to both. The integrated model was successfully benchmarked against multiple data sets. As one example, the authors modeled the consequences of the purine analog allopurinol, used to treat high concentrations of uric acid; their conclusions demonstrated good agreement with long-term allopurinol dosing, even though the data used as input was limited to a single dose. These results highlight the ability of the merged models to provide mechanistic insights into complex disease states. *CG*

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